RNA ISOLATION USING THE QIAGEN RNEASY MIDI KIT
Animal Tissue Protocol*
***See the specialized protocol for heart, muscle and skin tissue.**

For use of the midi columns, the weight of the tissue should be less than 150 mg. Even though the kit states, up to 250 mg of tissue may be used with the midi kit, it has been our experience that the yield of total RNA can be reduced due to column overloading, even when splitting the sample over 2 columns as we routinely do in the NMC. Read notes before proceeding with protocol. Tissues utilized in this protocol include liver, kidney, thymus, lung, prostate and ovary.

Tissue Preparation for Homogenization:

Tissue preparation for homogenization is done at room temperature under a hood.

1. Lysis buffer is prepared by adding 10 ul beta-mercaptoethanol ß-ME) per ml of RLT buffer. Four mls of ß-ME/RLT is required for each sample.
2. Tissue is quickly weighed (>20-150 mg) and placed in a medium-sized weigh boat containing ß-ME/RLT buffer (1 ml) and minced using 2 sterile razor blades for tissue disruption.
3. Pour the remaining ß-ME/RLT (3 ml) in a 15 ml round-bottom polypropylene tube (not provided). Add the minced sample and homogenize for a total of 60 seconds. (See tips for optimal homogenization.) Proceed to the next sample.

RNA Extraction:

All steps for the extraction of RNA are performed at room temperature. All centrifugations are carried out using a swinging bucket rotor at 4000 x g (the maximum speed of 3500-5000 rpm corresponds to 3000-5000 x g for most rotors).

4. After all the samples have been disrupted, centrifuge the tissue lysate for 5 min. Carefully pipette the supernatant (3.5 ml) into a new vessel (15-ml polypropylene tube, not provided). Make sure to place the pipet tip below the fatty upper layer, if present, and avoid pipetting the pellet at the bottom of the tube. Proceed to step 5.
5. Add one volume (3.5 ml) of 70 % ethanol to the homogenized lysate. ****For liver, use 50% ethanol.**** For all other tissues use 70% ethanol. Shake vigorously by inverting the tube 8-10 times. If a visible precipitate forms, re-suspend the precipitate by vigorous shaking (inverting the tube 8-10 times) and immediately proceed to the next step.
6. Split sample (3.5 ml) over **2 RNA easy midi spin columns** sitting in a 15-ml centrifuge tubes (provided) and close the tube gently by placing the cap on top of the tube. Centrifuge for 10 min. Discard flow through. Reuse collection tube in step 7.
7. Add 4 ml Buffer RW1 to the column and close the tube gently. Centrifuge for 5 min. to wash the column. Discard flow through. Reuse collection tube in step 8.
8. Add 2.5 ml Buffer RPE to the column and close the tube gently. Centrifuge for 2 min. Discard flow through. Reuse collection tube in step 9.
9. Add another 2.5 ml of Buffer RPE to the column and centrifuge for 10 min. to dry.
10. To elute RNA transfer spin column to a new 15 ml collection tube (provided). Pipet 200µl of RNase-free water directly onto the column membrane. Close tube gently, let it stand for 1 min then centrifuge for 3 min.
11. Repeat step 10 as described with a second volume of RNase-free water. Gently mix and measure volume of eluate.
12. Quantitate, concentrate and store RNA in a microcentrifuge tube at -70°C.

For NIEHS In-House Produced Arrays:
Aliquots of 100µg should be stored at 2µg/µl in RNase-free water.

For Agilent Commercial Arrays:
Aliquots of 60µg should be stored at 1.1 – 5.0µg/µl in Rnase-free water.

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